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Award Number: DAMD17-99-1-9014

TITLE: Identification of Cellular and Molecular Markers of Prostate Cancer Progression in Racial-Ethnic Minorities

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REPORT DATE: May 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

Form Approved
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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2002	Final (1 Nov 98 - 30 Apr 02)

4. TITLE AND SUBTITLE
Identification of Cellular and Molecular Markers of Prostate Cancer Progression in Racial-Ethnic Minorities

5. FUNDING NUMBERS
DAMD17-99-1-9014

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
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13. ABSTRACT ***(Maximum 200 Words)***
Our specific aims were to examine men with prostate cancer from 4 different racial-ethnic groups (African-American, Latino, Asian and Caucasians men) to determine the prevalence of molecular and cellular changes that may play a role in prostate tumor progression, particularly invasion and metastasis. The assessment of the tissue samples includes markers that are involved in the following pathways: a) cell cycle regulation, b)apoptosis, d) invasion and metastasis, and hormonal regulation.
Our work in the development of Spectral Imaging techniques will enable us to assess many more markers on the tissue available to us for this project than we had anticipated at the onset of this study. This will greatly enhance our understanding of the molecular and cellular changes in these tumor samples.
The results of the analysis of these markers are being assessed to determine the relationship between the changes in these key biological pathways and race/ethnicity, age and the intermediate markers of disease progression (tumor stage and Gleason grade). Finally, after sufficient follow-up time as elapsed, these changes will be related to clinical outcome (recurrence and survival) within and between the racial/ethnic groups.

14. SUBJECT TERMS
prostate cancer, racial-ethnic groups, tumor progression, molecular and cellular changes, metastasis

15. NUMBER OF PAGES
21

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Prostate cancer continues to be a major health risk for men and is, in fact, the most frequently diagnosed cancer in the United States, having surpassed female breast cancer in 1994. Although the epidemiology and etiology of prostate cancer is largely unknown, it is a disease with extraordinary racial-ethnic variation in incidence, mortality, and survival. African-American men have by far the highest rates of prostate cancer in the world, whereas Asian men native to China, Japan and Korea have the lowest. Even for prostate cancers presenting at a specific stage, African-Americans have substantially worse survival, whereas Asian-Americans appear to have substantially better survival than whites including Hispanics. Indeed, a recent report shows that even in an equal-access medical care setting, prostate cancer survival for black men is poorer compared to white men, suggesting that the disease is particularly aggressive in black men.

Although, the epidemiology and etiology of prostate is largely unknown it is known that it is an extremely heterogenous disease with an unpredictable course. Nevertheless, the steps that a tumor must undergo to be invasive and metastatic (i.e. the critical factors leading to patient death) are becoming increasingly well characterized. This study looked at some of the most important markers in prostate cancer in men from different racial-ethnic groups and analysis is continuing (under another DOD funded project (DAMD-17-00-1-0102) to determine the biologic significance of these markers. The major factors of interest include:

- Loss of hormonal regulation that can also have important implications in the control of metastatic disease.
- Loss of cell cycle control: Loss of tumor suppressor function (e.g. p53, Rb, PTEN) that can have multiple effects on regulation of cell growth, angiogenesis, and the ability of a tumor to enter the cell death (apoptotic) pathway. Similarly, inactivation of cdk-inhibitors (p27, p21, p16) is expected to result in increased proliferation rates of tumor cells (as detected by PCNA, Ki67 and Topoisomerase II (expression).
- Loss of growth control: In the last year a number of groups have identified loss of function of the PTEN phosphatase as a common event, particularly in advanced prostate cancer. The primary consequence of loss of PTEN function is deregulation of the PI3-kinaseAkt pathway, which is oncogenic in many tumor models. By measuring the status of this pathway at multiple levels, we will define the frequency of this change in multiple ethnic groups.
- The ability to form a new blood supply (angiogenesis), which is important in delivering nutrients and removing waste from a tumor, and also in providing a route for tumor metastasis. Loss of normal inhibitors of angiogenesis (thrombospondin-1) can lead to increased neovascularization (detected by microvessel density).
- Loss of normal cell matrix adhesion properties and cell-cell interactions (including contact inhibition), which allow tumor cells to grow past normal cell density and to break away from their primary site and form occult metastases, or overt metastases.

BODY

We are constantly searching for new markers that will help us address the biovariability of tumors among members of different racial-ethnic groups. To this end we have identified two new markers to add to our test battery. These are COX-2 and Caveolin-1. COX-2 is an isoform of cyclooxygenase and is an enzyme that metabolizes arachidonate to prostaglandin G2 and then to prostaglandin H2. COX-2 activity has been implicated as an important factor in tumorigenesis. The model most studies is that of colorectal cancer. More than 80% of human colorectal cancers have increased levels of COX-2 mRNA, as do about 40% of colorectal adenomas (Eberhart et al 1994). COX-2 inhibitors (such as NSAIDS) exhibit dramatic anti-neoplastic activity in experimental models of colorectal cancer. These include colorectal cancer cells implanted into nude mice, colon tumor production in APC (adenomatous polyposis coli) mutant mice, and carcinogen-induced colon tumors in rats (Oshima et al 1996; Sheng et al, 1997; Kawamori et al 1998). Transfection of COX-2 into human colon cancer cells have shown that COX-2 is involved in a number of processes fundamental to tumor development-apoptosis, tumor invasion and metastasis, and angiogenesis (Tsujii and DuBois, 1995; Tsujii et al, 1997, 1998). COX-2 appears to regulate the expression of a large number of genes associated with these processes. We are testing the hypothesis that COX-2 expression in primary tumor is an important molecular pathway of carcinogenesis in humans. We are using a modified protocol of Masferrer et al (2000) to measure COX-2 expression in prostate cancer. This protocol has been tested and optimized by our laboratory and applied to patient samples to date. The second new marker we have added is Caveolin-1. Caveolins are major structural proteins of caveolae-specialized plasma membrane invaginations that are abundant in smooth muscle cells, adipocytes, and endothelium, and mediate signal transduction activities and molecular transport (Harder et al, 1997). Initial studies based on the work by Thompson and Yang et al on Caveolin-1 expression in a large number of primary and metastatic pairs of cell lines derived from the MPR model system. Their results indicated that cavelin-1 protein was elevated in metastasis-derived cells relative to their matched primary tumor counterparts (Yang et al 1998). A further study by this group indicated that Caveolin-1 levels as measured by IHC were different in African-American versus Non-Hispanic whites with prostate cancer (Yang et al 2002). We have obtained this antibody and have subjected it to our rigorous validation protocol and have successfully optimized the protocol. We have now applied this Caveolin-1 antibody to our clinical samples our multi-racial cohort and differences in Caveolin-1 expression are currently being assessed (Figure 1).

As the library of clinically significant markers continues to increase, and the amount of tissue for analysis remains static, we recognized the need to new technology to optimize the amount of tissue available. We have successfully done this in collaboration with George McNamara. In summary, we have developed a working , a multi-marker technique by which we can look at up to 4 or more different markers of biologic status on a single tissue section using spectral imaging techniques (Figures 2). In the future we will also employ fluorescent markers to increase the number of markers we can assess on a single slide to tissue to 7 or more, thus multiplying our resources significantly. Until this

technique was developed and optimized for use in our laboratory we were unable to test all of the markers proposed on the limited number of slides available to us.

These new markers and novel techniques to maximize available tissue will better enable us to determine the relationship between the changes in these key biological pathways and a) race/ethnicity, b) age, and c) intermediate markers of tumor progression (tumor stage and grade). We will eventually be in a position to eventually relate these changes to clinical outcome (survival and mortality across racial-ethnic groups).

KEY RESEARCH ACCOMPLISHMENTS/REPORTABLE OUTCOMES

- We received formalin-fixed, paraffin-embedded tissue from 239 cases of prostate cancer and entered these into the laboratory database providing them with a laboratory number. This number is linked in our database to the patient's study identification number.
- We have assessed 219 of these for the presence of tumor, for the percentage of tumor to normal prostate tissue and recorded the Gleason grade of the tumor in the slides provided. In most cases (>90%), sufficient tissue is available for immunohistochemical analysis.
- We have stained, reviewed and recorded the results on 173 of the 219 cases received with antibody against p27. We have stained the same 173 cases with antibody against COX-2 and have analyzed and recorded the data on 77 of these. The remaining will be analyzed within the next few weeks. The same 173 cases are also currently being analyzed for Caveolin-1 (Table 1).
- We will also examine other factors known to be involved in tumor progression in various cancers, including prostate cancer, and other factors that play a potential role in prostate cancer progression. These include bcl-2, E-cadherin p53, Rb, CD34, p21, p16, Ki67, PCNA, Topoisomerase-II and thrombospondin-1.
- The development of the multiple marker analysis by Spectral Imaging will allow us to do most, if not all, markers listed on virtually every tissue with sufficient tumor present and will allow us to determine the molecular basis for the racial/ethnic differences in prostate cancer progression and morality.

This project has accomplished a great deal of work in terms of accruing patient samples and assessing them for tumor markers, (see above and Table 1) but as importantly it has served as the springboard for another project that has been funded by the DOD (DAMD-17-00-1-0102) led by Dr Ronald Ross. This expanded project, in which the SEER cohort of patients has been added, has enabled us to substantially increase both the number of patients examined, and, with the newly developed technology, is enabling us to look at more markers, including very recently described ones such as Caveolin-1. Therefore, the important work done under this grant will continue and will be expanded even further under this additional DOD funding. These studies are expected to provide information leading to a better understanding of prostate cancer progression in men of different racial/ethnic groups. While our study will have emphasis on racial/ethnic variability, it will also address important issues concerning prostate cancer outcome for all men. Facts

that predispose one group of men to have more aggressive tumor, may be predictive of behavior of prostate cancer in all men. Our initial focus will be on known pathways of tumor progression, studying factors that have been shown to be important (or potentially important) predictors of prostate cancer behavior.
A manuscript for a peer review journal based on the results obtained is now in preparation.

CONCLUSIONS

This study is a molecular epidemiologic study designed to study prostate cancer progression. It is designed to specifically elucidate multi-ethnic differences in prostate cancer risk and progression. It takes an innovative approach to develop and apply novel biologic markers of prostate cancer progression. It will investigate understudied populations of contrasting risk.
We have been successful in obtaining a large number of patient samples from various racial/ethnic groups and assessed these markers for tissue suitability, Gleason Grade and with the previously mentioned markers. We have not yet unblended the samples as to patient race and intermediate markers of tumor progression as primary tissue analysis is still ongoing.

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APPENDIX

Table 1 Results for p27 and Cox-2

Lab#	p27Results%	p27ResultsIntensitity	p27Readby	p27Comments	Cox-2Results	Cox-2Readby
1	>50	1+	DH			
10				lymphoid tissue		
100	>50	2+	MG/DH		0	DH
101	10-50	1+	MG/DH		2+ diffuse	MG/DH
102	>50	2+			1+ focal	DH
103	>50	3+	MG/DH			
104	>50%	4+	MG/DH			
105	>50%	4+	MG/DH			
106	>50%	2+	MG/DH			
107	>50%	4+	MG/DH		0	DH
108	>50%	4+	MG/DH		0	MG/DH
109	>50%	1+	MG/DH		0	DH
11	10/50	1+	DH			
110	>50%	2+	MG/DH			MG/DH
110	>50%	2+	MG/DH	3 slides read F-12,F-9, F-10		
111	>50	4+	MG/DH			
112	<10%	1+	MG/DH		0	DH
113	>50%	2+	MG/DH		1+ diffuse	MG/DH
114	>50%	2+	MG/DH		0	DH
115	>50%	4+	MG/DH		0	DH
116	>50%	3+	MG/DH			
117	>50%	3+	MG/DH		0	MG/DH
118	>50%	3+	MG/DH			
119	>50%	2+	MG/DH			
12	>50	1-2+	DH			
120	>50%	2+	MG/DH		3+ diffuse	MG/DH

121	>50%	3+	MG/DH		
126	>50%	4	MG/DH		
127	>50%	2+	MG/DH		
128	>50%	1+	MG/DH	0	DH
13	>50	2-3+	DH	2+ diffuse	MG/DH
131	>50%	2+	MG/DH		
132	10-50%	2+	MG/DH		
133	>50%	2+	MG/DH	0	DH
134					MA/DH
135	>50%	3+	MG/DH		
137	>50%	3+	MG/DH		
138	>50%	3+	MG/DH		
139	>50%	4+	MG/DH	0	MG/DH
14	>50	1-2+	DH	2+ focal	MG/DH
141	>50%	2+	MG/DH		
142	>50%	3+	MG/DH		
143	>50%	3+	MG/DH		
144	10-50%	1+	MG/DH		
146	>50%	3+	MG/DH		
147	>50%	2+	MG/DH		
148	<10%	1+	MG/DH		
149	>50%	3+	MG/DH		
15	>50	1+	DH		
150	>50%	2+	MG/DH		
151	>50%	1+	MG/DH		
152	>50%	3+	MG/DH		
153	10-50	2+	MG/DH		
154	>50%	1+	MG/DH		
156	>50	3+	MG/DH		
157	>50	1+	MG/DH		
158	>50	3+	MG/DH		

159						MA/DH
16				insufficient tumor		
160	>50%	4+	MG/DH			
161	>50	3+	MG/DH		0	DH
162	>50%	2+	MG/DH			
163	>50	3+	MG/DH		0	MA/DH
164	>50	3+	MG/DH		1+ focal	MA/DH
165	>50	3+	MG/DH			
166	>50%	1+	MG/DH		0	DH
167	>50%	3+	MG/DH			
168	>50%	4	MG/DH			
169	>50	3+	MG/DH			
17	>50	3-4+	DH		1+ diffuse	MG/DH
170	>50	1+	MG/DH			
171	>50	3+	MG/DH			
172	<10	1+	MG/DH			
173				insufficient tumor		
174	>50%	1+	MG/DH			
175	>50	2+	MG/DH	repeated staining; initial results <10 0 (DH)	0	MG/DH
176				insufficient tumor		
177				no tumor		
178				no tissue		
179	>50%	4+	MG/DH			
18	>50	3+	DH		2+ diffuse	MG/DH
180	>50%	1+	MG/DH			
181	>50	1+	MG/DH		1+ focal	MG/DH

182	>50	1+	MG/DH		3+ diffuse	MG/DH
19	>50	1-2+	DH		1+ diffuse	MG/DH
2	>50	3+	MG/DH			
20	<10	0-1+	DH		0	MG/DH
21	<10	0-1+	DH			
22	<10	0-1+	DH		2+ diffuse	MG/DH
23	10-50 &>50	0-1+ and 2+	DH	2 slides were read with different results	3+ diffuse	MG/DH
24	<10	1+	MG/DH		no tumor re-stain	
25				insufficient tumor		
26	10-50	1+	DH			
27	10-50	3+	DH		0	DH
28	>50	1+	DH		0/0	MG/DH
29	>50	2+	DH		2+ diffuse	MG/DH
3	>50	3+	DH		3+ diffuse	MG/DH
30	>50	3+	DH		1+ diffuse	MG/DH
31	>50	2+	DH		0	MG/DH
33	10-50	1+	DH		2+ diffuse	MG/DH
34	>50	2+	DH		3+ diffuse	MG/DH
35	10-50	1+	DH			
36	10-50	1+	DH			
37	>50	4+	MG/DH			
38	>50	2+	DH			
39	<10	0-1+	DH			
4	<10	0-1+	DH		1+ diffuse	MG/DH

40	>50	3+	MG/DH	repeated stainig: initial results <10 0-1+ (DH)		
41	10-50	2+	MG/DH	repeated staining ; initial results <10 0-1+ (DH)		
42	>50	2+	MG/DH	Repeat-staining initially unsatisfactory	0/0	MG/DH
43	10-50	1-2+	DH			
5	>50	2+	DH			
51					1+ focal	MG/DH
52	>50	2-3+	DH		2+ diffude	MG/DH
54	>50	3+	MG/DH	repeated staining;initial <10 0+ (DH)		
55	<10	0	DH			
56	10-50	1+	MG/DH			
56	<10	0	DH		0	MG/DH
57	>50	2+	DH		0	MG/DH
58	>50	3+	DH			
59	<10	0-1+	DH			
6	>50	3+	DH			
60	10-50	1+	DH		3+/2	MG/DH
61	<10	0-1+	DH			
62						
63					1+ diffuse	MG/DH
64					1+ focal	MG/DH
65	>50	3+	DH			

66	<10	0-1+	DH			
67	>50	2-3+	DH		2+ diffuse	MG/DH
68	10-50	1+	DH		0	MG/DH
69	<10	0-1+	DH			
7	>50	1+	DH		0	DH
70	>50	32-+	DH	2 slides read, one 2+, the second 3+ intensity		
71	>50	3+	DH			
72	>50	3+	DH		0	MG/DH
74	>50	3+	DH			
75	>50	1+	DH		1 focal	MG/DH
76	>50	2+	MG			
78	>50	3+	MG			
79	<10	0-1+	MG		1+ diffuse	MG/DH
8	>50	3+	DH	Results same on two different blocks	3+ diffuse	MG/DH
80	10-50	3+	MG/DH		0/0	MG/DH
81	>50	2+	MG/DH	repeat p27 >50% 3+		
82				insufficient tumot on slide		
83	>50%	1+	MG/DH			
85	>50	2+	MG/DH	repeat p27 IT		
86	>50	2+	MG/DH			
87	>50%	3+	MG/DH			
88	>50	2+	DH			
89	>50	3+	MG/DH			
9	<10	0-1+	DH			

90	>50	2+	MG/DH			
91	>50	2+	MG/DH			
92	>50	4+	MG/DH			
93	>50	4+	MG/DH			
94	10/50	1+	MG/DH			
95	10-50	1+	MG/DH		1+ focal	MG/DH
96				insufficient tissue on slide		
97	>50	2+	MG/DH		1+ diffuse	MG/DH
98	>50%	2+	MG/DH			
99	<10%	1+	MG/DH		0	DH

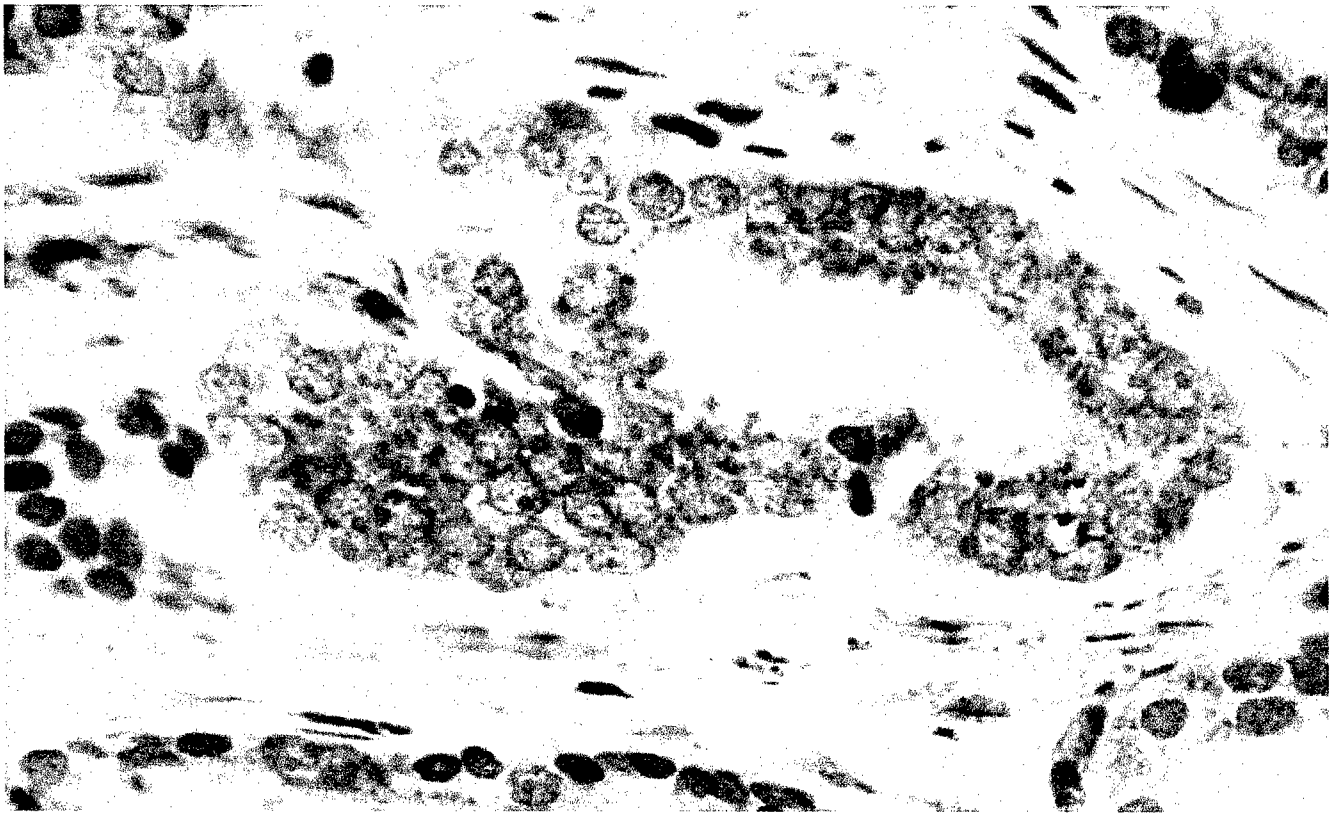

Figure 1: Formalin-fixed, paraffin-embedded prostate tissue form study cohort showing granular cytoplasmic Caveolin-1 immunoreactivity.

Figure 2
Spectral Imaging Analysis to discern multi-marker antigen staining

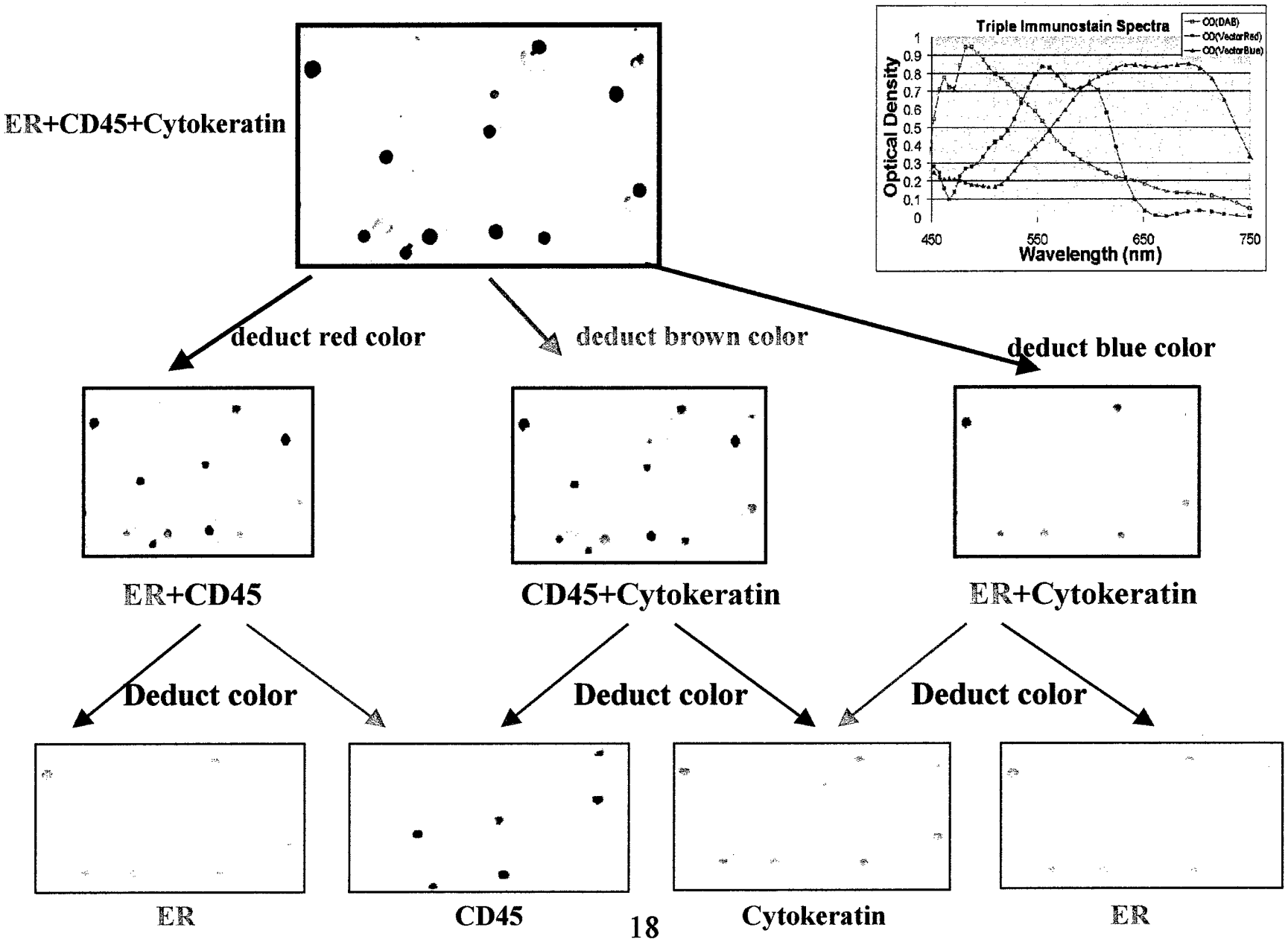

Figure 2 Legend: This figure demonstrates Spectral Imaging applied to a mixture of lymphocytes and breast cancer cells. Three markers were applied, Cytokeratin with Vector Red™ Vector Laboratories, Burlingame, CA) used as the chromogen , CD45 with Vector Blue™ (Vector Laboratories, Burlingame, CA) used as the chromogen and Estrogen Receptor (ER) with DAB (ScyTek, Logan UT) used as the chromogen. The Spectral analysis process can deduct a single marker by removal of the chromogen color (second row) or a single marker can be assessed by removing all other chromogens (third row). In this way the total number of cells positive for a given marker can be assessed regardless of what other markers (if any) may be present on the individual cells. Furthermore, co-expression of more than one marker on a single cell can be assessed. For example, in this example it can clearly be seen that cytokeratin and ER are expressed on the same cell populations, whereas, CD 45 is positive on a distinct population of cells.

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